Transplant regimens

Preconditioning consisted of cytarabine (Ara-C), busulfan (3.2 $mg \cdot kg^{-1} \cdot day^{-1}$ administered intravenously on days −8 to −6) (day 0 being the day of donor cell infusion), cyclophosphamide (1.8 $g \cdot m^{-2} \cdot day^{-1}$, days −5 to −4), and simustine (250 $mg \cdot m^{-2}$, day −3). Ara-C was administered at 4 $g \cdot m^{-2} \cdot day^{-1}$ (days −10 to −9) to the human leukocyte antigen (HLA)-haploidentical related donor (haplo-RD) group, at 2 $g \cdot m^{-2} \cdot day^{-1}$ (days −10 to −9) to the HLA-unrelated donor (URD) group, and at 2 $g \cdot m^{-2} \cdot day^{-1}$ (day −9) to the HLA-identical sibling donor (ISD) group. Rabbit anti-thymocyte globulin (thymoglobulin, 2.5 $mg \cdot kg^{-1} \cdot day^{-1}$, days −5 to −2; Sanofi, France) was administered to the haplo-RD and URD groups. Granulocyte colony-stimulating factor (G-CSF)-mobilized, fresh and unmanipulated bone marrow (BM) and peripheral blood harvests were administered to the recipients the day they were collected. In addition, patients received cyclosporine A (CSA), mycophenolate mofetil (MMF), and short-term methotrexate (MTX) as graft-versus-host disease (GVHD) prophylaxis agents.

MRD-directed immunotherapy protocol

Patients with a single BM sample tested positive for LAIPs or *WT1* should receive IFN-α treatment, and 18 patients agreed to receive IFN-α treatment. For the patients who did not agree to receive any interventions, the tests were repeated 2 weeks after positive results for *WT1* or LAIPs results were obtained (n=19). If 2 consecutive BM samples tested positive for LAIPs or *WT1* expression within a 2-week interval, patients should receive MRD-directed intervention, and those who could not receive DLI, because of patient refusal (n=10) or provider refusal (n=2), received IFN-α treatment. Cases in which a single BM sample tested positive for both *WT1* expression and LAIPs received IFN-α treatment if they could not receive DLI because of patient refusal (n=3) (Figure 1). MRD status was also monitored 1, 2, 3, 4.5, 6, 9, and 12 months after MRD-directed immunotherapy and at 6-month intervals thereafter.

Cases in which a single BM sample tested positive for LAIPs or *WT1* expression were defined as MRD_{sin+}. Cases in which 2 consecutive BM samples within a 2-week interval tested positive for LAIPs or *WT1* expression, or those in which a single BM sample tested positive for both LAIPs and *WT1* expression were defined as MRD_{co+}. MRD-directed Chemo-DLI was the first choice for patients in MRD_{co+} group and those who could not receive DLI because of patient or provider refusal received IFN-α treatment.

Patients with active GVHD, active infections, severe myelosuppression, organ failure, or relapse were excluded from MRD-directed IFN-α treatment. Recombinant human IFN-α-2b injections (Anferon; Tianjin Hualida Biotechnology Co., Ltd., Tianjin, China) were subcutaneously administered for 6 cycles (twice or thrice weekly every 4 weeks) at 3 million units (MU) for patients over 16 years and at 3 MU/m^2 for those under 16 years (capped by 3 MU). Prolonged treatment with IFN-α was permitted at patients' request. IFN-α was suspended in all patients with severe GVHD, severe infection, grade ≥3 toxicity, relapse, or non-relapse mortality (NRM).

Patients with active GVHD, active infections, organ failure, or relapse were excluded from MRD-directed Chemo-DLI treatment. The dose of mononucleated cells (MNCs) for Chemo-DLI was 1-2×10^8 MNCs/kg. DLI doses were also defined as the number of $CD3^+$ cells per kilogram of recipient weight (range, 1.0× 10^7/kg to 10.0 × 10^7/kg). Patients received anti-leukemic chemotherapy 48–72 hours before DLI. Chemotherapy regimens included HAA (harringtonine 2 mg·m^{-2}·day^{-1} for 5 days, aclacinomycin 10 mg·m^{-2}·day^{-1} for 5 days, and cytarabine 100 mg·m^{-2}·day^{-1} for 5 days; n=4) and AA (aclacinomycin 10 mg·m^{-2}·day^{-1} for 5 days and cytarabine 100 mg·m^{-2}·day^{-1} for 5 days; n=10). All Chemo-DLI recipients received short-term immunosuppressive agents after DLI, which could reduce the incidence and severity of DLI-associated aGVHD without influencing the GVL effect. Patients receiving Chemo-DLI from an ISD received GVHD prophylaxis for 4–6 weeks, while those receiving Chemo-DLI from a haplo-RD received GVHD prophylaxis for 6–8 weeks at the discretion of the attending physicians (and usually depending on the patient's GVHD status after Chemo-DLI). The starting dosage of CSA was 2.5 mg·kg^{-1}·day^{-1}, which was later adjusted to maintain a plasma concentration >100 ng/mL. MTX was administered at 10 mg intravenously on days 1, 4, 8, and weekly thereafter for 2–6 weeks.

Treatment of GVHD after MRD-directed IFN-α treatment

The treatment of GVHD was in accordance with the common international criteria. Acute GVHD (aGVHD) was treated with methylprednisolone (1–2 mg·kg^{-1} per day) and by resumption of full-dose CSA administration. Second- or third-line immunosuppressive therapies such as CD25 monoclonal antibody (Basiliximab; Novartis Pharma Stein AG, Basel, Switzerland), MMF, tacrolimus, or MTX were administered in cases of steroid-refractory aGVHD. Moderate to severe chronic GVHD (cGVHD) was treated with prednisone (1 mg·kg^{-1} per day), and CSA was adjusted to maintain a blood

concentration >150 ng/mL. Second- or third-line immunosuppressive therapies such as MMF, MTX, penicillamine, azathioprine, or tacrolimus were administered in cases of steroid-refractory cGVHD.